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Morphological Observations on Male Nematodes of the Subfamily Ostertagiinae in Capive Chinese Water Deer (*Hydropotes inermis*: Artiodactyla: Mammalia) at Whipsnade Wild Animal Park, UK

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Introduction

Gastrointestinal nematodosis caused by trichostrongylids (Trichostrongyloidea: Trichostrongylidae) has been recognized as an important disease of captive wild ruminants [10, 14, 23]. However, despite the importance of this disease few studies on nematode parasites of captive wild ungulates have been reported [27]. This may be in part due to the lack of expertise available to assist in the identification of wild animal parasites [14]. Although there are now molecular assays for the identification of economically important gastrointestinal nematodes of cattle [38], the identification of nematode parasites of wild animals still relies upon morphological examination [6, 30] because to date few molecular studies of wild animal nematode parasites have been published. The present study was therefore designed to add to our knowledge of nematode parasites of captive Chinese Water Deer (*Hydropotes inermis*) focussing in particular upon two ostertagiine species that exhibit polymorphism in domestic ruminants. The overall objectives of the present study were to:

(i) identify the genera and species of abomasal nematodes recovered at post mortem from Chinese Water Deer at Whipsnade Wild Animal Park;

(ii) compare the relative numbers of *Ostertagia leptospicularis* and *Skrjabinagia kolchida* in each host; and

(iii) evaluate the measurement of the proconus as an aid to the identification of *O. leptospicularis* from *O. ostertagi*.

Materials and Methods

The study was conductd at Whipsnade Wild Animal Park (WWAP), opened in 1931 by the Zoological Society of London. WWAP which is a 265 hectare zoological collection in Bedfordshire (at 51.8° N, 0.5°W), UK, specializing in exhibiting and breeding herd animals, especially ruminants, in large grass enclosures and has been highly successful in breeding many, often endangered, species. Gastrointestinal parasitism of the ruminants is an important cause of disease and mortality at the Park and has been the subject of a number of investigations [7, 9, 11, 16, 17, 22, 27, 29].

The nematodes examined in this study were recovered during the post mortem examination of Chinese Water Deer, that had been found recently dead or that had been euthanased at WWAP between January 2000 and February 2001, by the veterinary officer Dr. E. J. Flach. The present study included 12 Chinese Water Deer details of which are given in Table 1.

Nematodes were collected by opening the abomasum of individual Chinese Water Deer along its greater curvature, collecting the contents in a bucket and washing the mucosa under a stream of tap water to facilitate the recovery of adult stages. Abomasal washings were made up to ca. 10 L, an aliquot (2%) withdrawn and formaldehyde added to give a final concentration of 5% prior to storage and subsequent examination [9]. Abomasal samples were examined in a petri dish under a dissecting microscope and all the nematodes were removed and counted.

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Table 1 Chinese Water Deer examined at Whipsnade Wild Animal Park: background information.

ID No.	Clinical No.	Age	Weight (kg)	Sex	Found dead	PM Date	remarks
1	XR47	old mature	7.6	male	28/Sep. /2000	28/Sep. /2000	found dead
2	XS7	young adult	6.1	male	3/Jan. /2001	4/Jan. /2001	found dead
3	XS16	adult	5.8	male	Eu. 6/Feb. /2001	8/Feb. /2001	found collapsed and very thin, euthanased
4	XS18	adult	7.5	male	12/Feb. /2001	13/Feb. /2001	——
5	XR2	adult	8.0	male	12/Jan. /2000	13/Jan. /2000	found dead
6	XS6	adult	6.6	female	3/Jan. /2001	4/Jan. /2001	found dead with very poor condition
7	XR52	subadult	5.7	male	23/Nov./2000	23/Nov./2000	found dead with poor condition
8	Data not available (Institute of Zoology reference no. 18)						
9	Data not available (Institute of Zoology reference no. 19)						
10	Data not available (Institute of Zoology reference no. 23)						
11	Data not available (Institute of Zoology reference no. 25)						
12	Data not available (Institute of Zoology reference no. 27)						

All mature male nematodes were isolated from fixed samples, mounted on microscope slides and were cleared in lactoglycerole. A coverslip was then placed on top of each slide in prior to detailed examination. The nematodes were then identified microscopically using standard taxonomical keys [30] and photographed where appropriate. After identification, the following measurements were made for each male *Ostertagia* and *Skrjabinagia* worm: body length, spicule length, oesophageal length, proconus (ventral swelling of the genital cone) height, Sjoberg's organ (where present) and bursa (Fig. 1). These features were measured using an eyepiece graticule mounted in the 10X eye piece and 4X, 10X, and 40X objectives, calibrated initially using a stage micrometer. Each small division of the eyepiece graticule represented 12.5μm using the X4 objective, 5μm using the X10 objective and 1.25μm using the X40 objective lens.

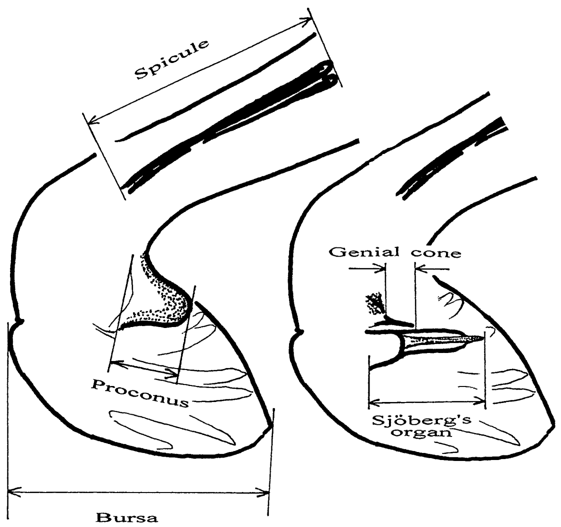

Fig. 1 Diagram showing proconus (or genital cone) and Sjoberg organ in a male *Skrjabinagia kolchida*. Note: Sjober's organ is not presnt in *Ostertagia* species.

Results

Nematode genera and species. A total of 701 nematodes recovered from Chinese Water Deer were examined in this survey. These belonged to six species, i.e. *Camelostrongylus mentulatus* (abbreviated to Cm in Table 2), *Ostertagia leptospicularis* (Ol), *O. ostertagi* (Oo), *Skrjabinagia kolchida* (Sk), *Spiculopteragia asymmetrica* (Sa) and *Trichostrongylus axei* (Ta).

Number of worms. The number of male worms examined from each deer is shown in the Table 2. Among the worms, *Ostertagia leptospicularis* and *Skrjabinagia kolchida* were not only the most numerous (total 87 and 48 individuals, respectively) but also the most prevalent (92% and 83%, respectively) nematode species recovered. Behind the result, there are intensity (total 19 individuals) and prevalence (67%) of *O. ostertagi*. Number and prevalence of other parasite were between 14 and 17, and between 25% and 42%, respectively.

Ratio of *Ostertagia leptospicularis* : *Skrjabinagia kolchida*. The number of *O. leptospicularis* and *S. kolchida* obrained from each Chinese Water Deer and thence the ratio of the two species are given in Table 3. According to the table, prevalence figures were more evenly balance at 64.6% *O. leptospicularis*: 35.4% *S. kolchida*.

Table 2 Nematode examined from individual of Chinese Water Deer.

ID Nos.	Cm	Ol	Oo	Sk	Sa	Ta	non-ident. males*	Females	TOTAL
1	0	44	8	27	0	9	4	138	230
2	0	4	1	3	3	1	0	22	34
3	0	8	1	3	5	0	0	31	48
4	11	4	3	1	2	4	0	41	66
5	0	8	1	2	0	0	0	45	56
6	0	2	0	2	4	2	1	21	32
7	3	4	2	1	0	1	0	23	34
8	0	1	0	0	0	0	0	11	12
9	2	10	2	6	0	0	0	69	89
10	0	1	0	2	0	0	0	32	35
11	0	1	1	0	0	0	0	27	29
12	0	0	0	1	0	0	0	35	36
Total	16	87	19	48	14	17	5	495	701
Prevalence in the present hosts examined**									
	25	92	67	83	33	42	–	–	–

* : *Ostertagia* sp. including L5.

**: Nematode positive number of the deer/ Total number of the deer examined X 100 (%).

Abbreviations. Cm: *Camelostrongylus mentulatus*, Ol: *Ostertagia leptospicularis*, Oo: *O. ostertagi*, Sk: *Skrjabinagia kolchida*, Sa: *Spiculopteragia asymmetrica*, Ta: *Trichostrongylus axei*.

Table 3 Ratio of *Ostertagia leptospicularis* and *Skrjabinagia kolchida* obtained from individual Chinese Water Deer.

ID Nos.	Ol	Sk	Ol+Sk
1	44 (62.0%)	27 (38.0%)	71
2	4 (57.1%)	3 (42.9%)	7
3	8 (72.7%)	3 (27.3%)	11
4	4 (80.0%)	1 (20.0%)	5
5	8 (80.0%)	2 (20.0%)	10
6	2 (50.0%)	2 (50.0%)	4
7	4 (80.0%)	1 (20.0%)	5
8	1 (100.0%)	0 (0.0%)	1
9	10 (62.5%)	6 (37.5%)	16
10	1 (33.3%)	2 (66.7%)	3
11	1 (100.0%)	0 (0.0%)	1
12	0 (0.0%)	1 (100.0%)	1
Range	0-44	0-27	
Mean	7.3 (64.6%)	4.0 (35.4%)	11.3 (100%)
SD	12.01	7.42	
SEM	12.01/3.46=3.47	7.42/3.46=2.14	
95%CI	0.5-14.1	-0.19-8.19	

Measurements of *Ostertagia* spp. and *Skrjabinagia kolchida*. In the present study, approximately 17.9% of male *Ostertagia* spp. nematodes observed were identified as *O. ostertagi*. Two *Ostertagia* species recorded in the present study may be differentiated by examination of the proconus which is well-developed (with an acute-angled bursa; Fig. 2) in the case of *O. leptospicularis* and less well developed (with a rounded bursa; Fig. 5) in the case of *O. ostertagi* (see Fig. 9). However, in this study, bursal shape and proconal size was somewhat variable. For example, eg., presences of individuals of *O. leptospicularis* with slightly lower proconus within well-developed bursa (Fig. 3), or with proconus of the *"leptospicularis"* type within slightly rounded bursa (Fig. 4). On the other hand, there are several individuals of *O. ostertagi* with relatively

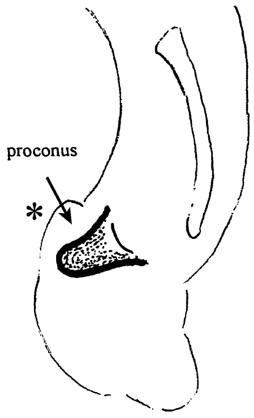

Fig. 2 Posterior extremity of male of *Ostertagia leptospicularis*, left-lateral view, showing a well-developed procpnus and acute-angled bursa (*). Nematode ID.-No. 44 (cf. appendix 1).

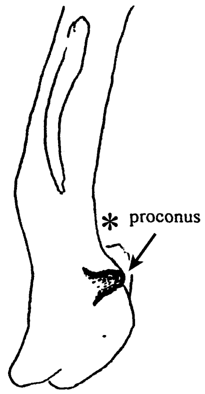

Fig. 4 Posterior extremity of male of *Ostertagia leptospicularis*, right-lateral view, showing a well-developed procpnus and slightly rounded bursa (*). Nematode ID.-No. 3 (cf. appendix 1).

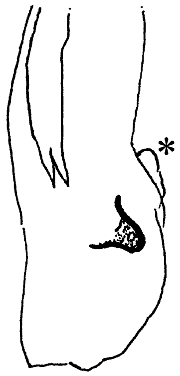

Fig. 3 Posterior extremity of male of *Ostertagia leptospicularis*, right-lateral view, showing a slightly lower proconus and acute-angled bursa (*). Nematode ID.-No. 95 (cf. appendix 1).

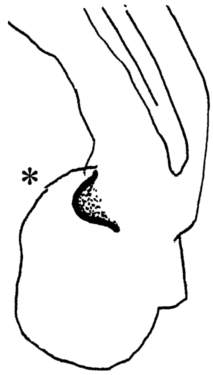

Fig. 5 Posterior extremity of male of *Ostertagia ostertagi*, left-lateral view, showing lower and rounded proconus, and more rounded bursa (*). Nematode ID.-No. 85 (cf. appendix 1).

developed proconus (Fig. 6) and/or bursal lobe (Fig. 7). Hence, the measurements of height of proconus and bursa with body, spicule and oesophagus of *Ostertagia* spp. including *Skrjabinagia kolchida*, which is considered as morphotype of *O. leptospicularis* were done.

Each measurement was shown in appendixes 1 and 2, and its statistical comparisons and relationship between the data were made as shown in the Table 4 and Figures 10–14. In general, height of proconus of *O. leptospicularis* was larger than one of *O. ostertagia*, although there is overlap between both ranges (Tab. 4, Figs. 10 and 15). Bursal height is almost constant in *Ostertagia* spp., although bursa of *Skrjabinagia kolchida* is slightly larger than ones of *Ostertagia* spp. (Tab. 4, Fig. 12). *Skrjabinagia kolchida* is easily differenciated from *Ostertagia* spp. because of presence of Sjoberg's organ (Figs. 1 and 8). And, there is a remarkable variation of the heights of genital cone and Sjoberg's organ. On the other hand, there is also no evident differences in the values of the measurements of body, spicule and oesophagus between *Ostertagia* spp. (Tab. 4, Figs. 11 and 13). However, it was cleared that the values of the measurements, especially oesophagus length and bursal height, of *S. kolchida* is longer and/or larger than ones of *Ostertagia* spp. (Tab. 4; Figs. 11–13).

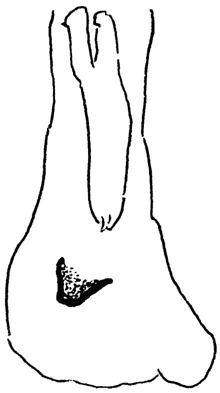

Fig. 6 Posterior extremity of male of of *Ostertagia ostertagi*, left-lateral (slightly sub-dorsal) view showing slightly developed proconus. Nematode ID.-No. 2 (cf. appendix 1).

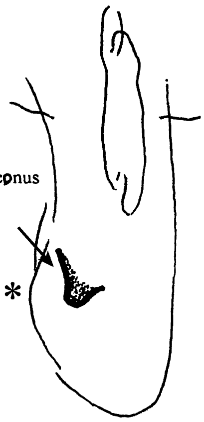

Fig. 7 Posterior extremity of male of *Ostertagia ostertagi*, left-lateral (slightly sub-dorsal) view, showing slightly longer bursa (*). Nematode ID.-No. 6 (cf. appendix 1).

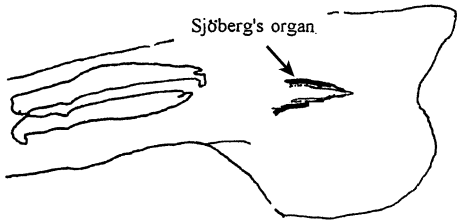

Fig. 8 Posterior extremity of male of *Skrjabinagia kolchida*, sub-ventral dorsa view. Nematode ID.-No. 102 (cf. appendix 2).

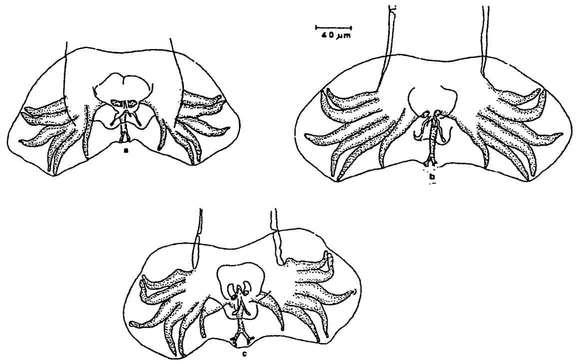

Fig. 9 Comparative morphology of caudal bursas (ventral views) of *Ostertagia ostertagi* (a), *O. leptospicularis* (c), and their hybrid (b) [34].

Discussion

Chinese Water deer, the only species found in the genus *Hydropotes*, occurs in Korea and in eastern China [26]. The head and body length is approximately 850 mm, tail length 70 mm, shoulder height 500 mm and body weight 30kg. This cervid species lives among tall reeds and rushes along rivers and also frequents tall grass areas on mountains and cultivated fields. In the wild Chinese Water Deer have been hunted for their colostrum used in folk medicine and also because they are considered to be an agricultural pest. This has resulted in the species being classified as near threatened by the IUCN. Fortunately, Chinese Water Deer have also been bred successfully in captivity, including the herd established at Whipsnade Wild Animal Park. There have been few reports describing the parasites found in Chinese Water Deer. The trematode families, Dicrocoeliidae and Paramphistomatidae, were reported in wild Chinese Water Deer in China [35, 37]. In Britain, Corbet and Harris [4] and Ohira et al. [27] described finding the nematodes *Camelostrongylus mentulatus, Ostertagia leptospicularis, O. ostertagi, Skrjabinagia* (=*Ostertagia*) *kolchida, Spiculopteragia asymmetrica* and *Trichostrongylus axei* in the abomasum, *T. colubriformis* in the small intestine and *Oesophagostomum venulosum* in the large intestine. Previous work [27] at WWAP revealed the prevalence of abomasal nematodes in 14 Chinese Water Deer to be *Ostertagia leptospicularis* and *Skrjabinagia kolchida* (43%), *O. ostertagi* (14%), *Camelostrongylus mentulatus, Spiculopteragia asymmetrica* and *Trichostrongylus axei* (7%). In general, members of the genus *Trichostrongylus* are found in a wide range of domestic and/or wild ungulates and

Table 4 Summary of morphological measurements of *Ostertagia* spp. and *Skrjabinagia kolchida* obtained from Chinese Water Deer.

	O. ostertagi	O. leptospicularis	S. kolchida
Body Range	4.6mm-6.9mm (n=18)	3.0mm-7.3mm (n=84)	3.8mm-7.8mm (n=47)
Mean	5.81	6.02	6.35
SD	0.826	0.911	0.995
SEM	0.19	0.10	0.15
95%CI	5.44-6.18	5.82-6.22	6.06-6.64
Spicule Range	145um-225um (n=19)	145um-190um (n=85)	140um-225um (n=48)
Mean	166.0	164.5	169.5
SD	19.55	12.90	20.4
SEM	4.40	1.40	2.94
95%CI	157.4-174.6	161.8-167.2	163.74-175.3
Oesophagus Range	500um-825um (n=18)	525um-975um (n=84)	500um-913um (n=45)
Mean	722.9	762.5	783.8
SD	102.65	73.38	78.63
SEM	24.20	8.00	11.72
95%CI	675.5-770.3	746.8-778.2	760.8-806.8
Proconus or genital cone Range	19um-33um (n=19)	28um-56um (n=85)	13um-69um (n=24)
Mean	26.9	39.3	24.4
SD	4.03	4.66	11.64
SEM	0.92	0.51	2.99
95%CI	25.1-28.7	38.3-40.3	18.5-30.26
Sjoberg's organ Range	——	——	38um-138um (n=48)
Mean	——	——	84.5
SD	——	——	25.11
SEM	——	——	3.62
95%CI	——	——	81.4-87.6
Bursa Range	125um-175um (n=14)	125um-190um (n=79)	135um-250um (n=43)
Mean	148.5	151.5	180.5
SD	15.35	15.70	26.90
SEM	3.62	1.77	4.10
95%CI	141.4-155.6	148.0-155.0	172.5-188.5

lagomorphs (accidentally, rodents) [1, 30, 32] though tend to be of low pathogenecity in temperate regions [36].

With the exception of *T. axei*, the nematodes found in Chinese Water Deer belong to the subfamily Ostertagiinae, The taxonomy and systematics of the Ostertagiinae, which includes between 7 and 17 genera [14] depending upon the status afforded to strains, polymorphism, species complexes and hybridization [15], is still been subject to much debate. Among them, the polymorphism hypothesis was based on the following observations [14, 15, 18]: (i) pairs of male morphotypes consistently occur together, with one constituting a "major" proportion and the other a "minor" proportion of the combined population; and (ii) consistent structural differences allow recognition of each of the morphological types. In the past this led to the recognition of separate genera and species for major and minor morphotypes. The proposal for polymorphism has been corroborated based on morphological, biochemical, and molecular ground [19–21].

Despite this debate, the species belonging to this subfamily seem to fall naturally into one of two groups [6] (i) *Ostertagia sens. lat*., parasites of Bovidae, originating from parasites of lagomorphs; and (ii) *Spiculopteragia sens. lat*., parasites of Cervidae, originating from parasites of suids and tragulids. The ostertagiines are characterized by having a reduced buccal capsule and a well-developed copulatory bursa in the male. Cervical papillae are prominent, and the synlophe [6] is composed of a large number of cuticular ridges that are perpendicular to the body surface. The genital cone, especially when swollen, is called a

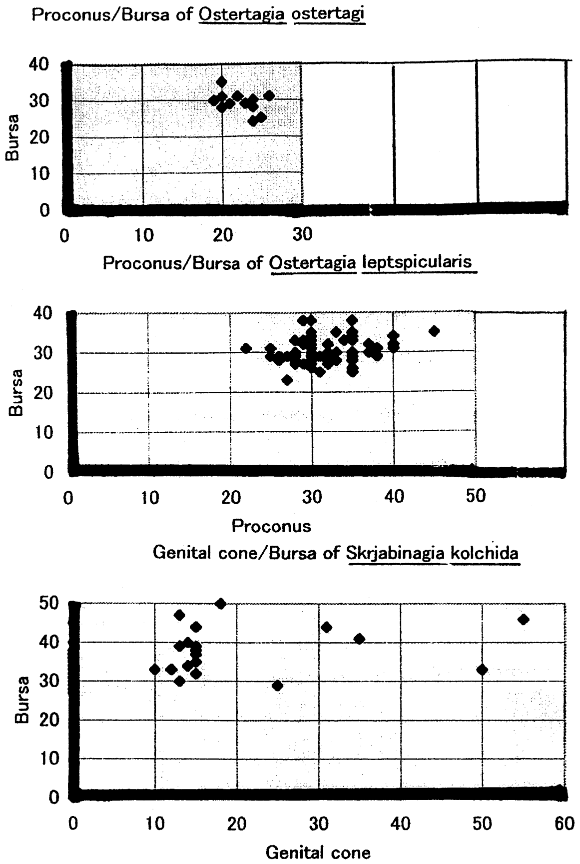

Fig. 10 Relationship between proconus (or genital cone) and bursa of *Ostertagia* spp. and *Skrjabinagia kolchida* obtained from Chinese Water Deer.

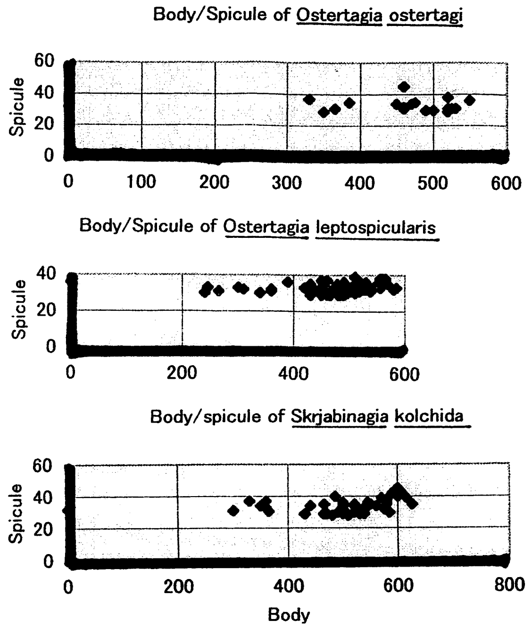

Fig. 11 Relationship between body and spicule of *Ostertagia* spp. and *Skrjabinagia kolchida* obtained from Chinese Water Deer.

"proconus" [15, 21]. The lateral rays of the bursa are in a pattern of 2-1-2 or 2-2-1 [6]. Ientification of this nematode group is therefore based on the structure of the bursa, genital cone, and spicules in males and dimensions of the oesophageal valve and the configuration of the synlophe [6, 15, 30] in the female.

The life cycles of the ostertagiines is direct, i.e. adult worms reside in the abomasum, embryonated eggs are passed in feces, and the first to third larval stages are free-living. The infective third-stage is ensheathed, and parasitic development and the prepatent period require between 2 and 3 weeks. Early fourth-stage larvae may be retained in the abomasal mucosa for extended periods of time prior to resuming maturation to the adult stage, a phenomenon known as hypobiosis [14].

The ostertagiines are among the most pathogenic of the strongyles known in ruminants [14], the most marked changes occurring as the fifth larval stage (or young adult) emerges from the abomasal glands [2, 31, 36].

Of the nematodes recorded from the Chinese Water deer, the genus *Ostertagia* is the most pathogenic [2, 25, 31, 36]. *Skrjabinagia kolchida* is considered a minor pathogen of cattle [36] and, in addition, regarded as the "minor morphotype" of *Ostertagia leptospicularis* [14, 18] and so its occurrence in captive or wild ruminants is of potential significance from the epidemiological point of view.

Ostergiinae other than *Ostertagia* and *Skrjabinagia* in Chinese Water deer include the following two nematodes:

(i) *Camelostrongylus mentulatus*.

Although this nematode has been associated with a wide range of artiodactyle hosts, especialy the Camelidae and Bovidae [8, 27, 29], it has been reported in several species of Cervidae including red deer [7, 12, 27, 29]. The nematode causes ostertagid-like lesions in the abomasum of sheep [13] and has been a major cause of mortality in Thomson's gazelles [17] and blackbuck [10] in

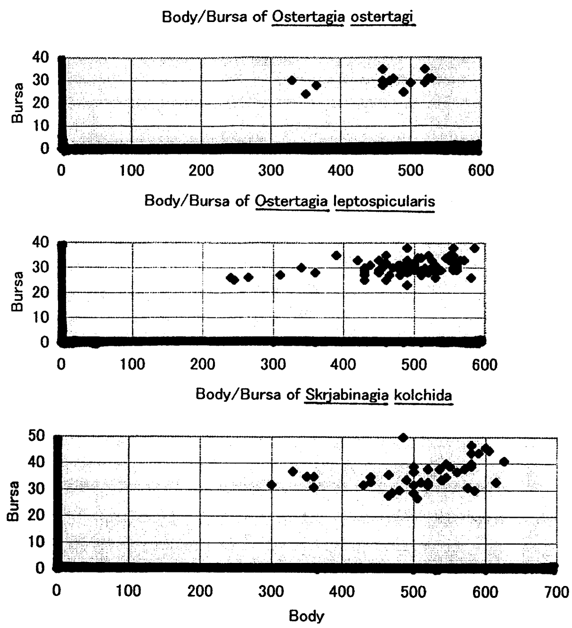

Fig. 12 Relationship between body and bursa of *Ostertagia* spp. and *Skrjabinagia kolchida* obtained from Chinese Water Deer.

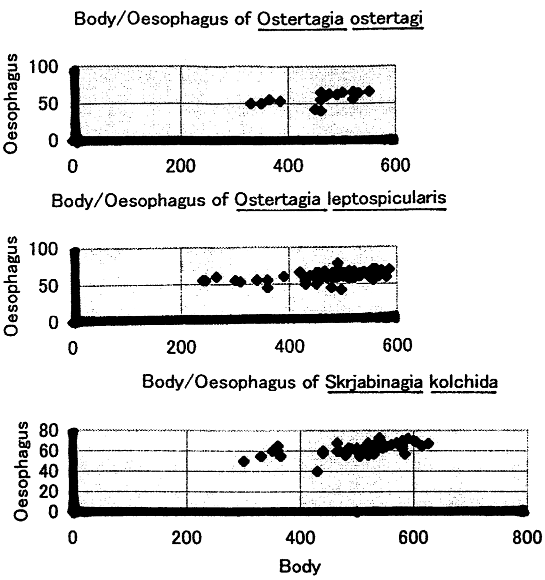

Fig. 13 Relationship between body and oesophagus of *Ostertagia* spp. and *Skrjabinagia kolchida* obtained from Chinese Water Deer.

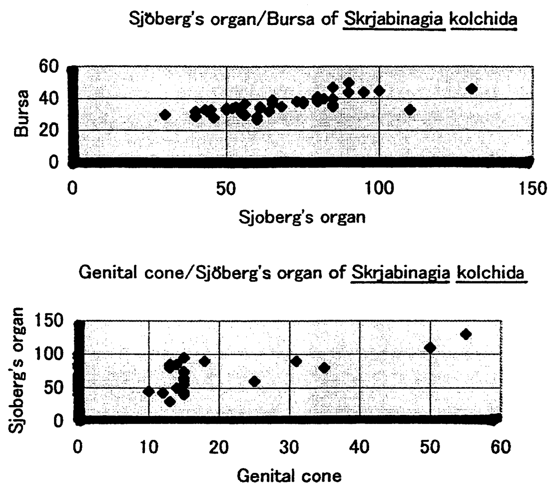

Fig. 14 Relationship between Sjoberg's organ and genital cone or bursa of *Skrjabinagia kolchida* obtained from Chinese Water Deer.

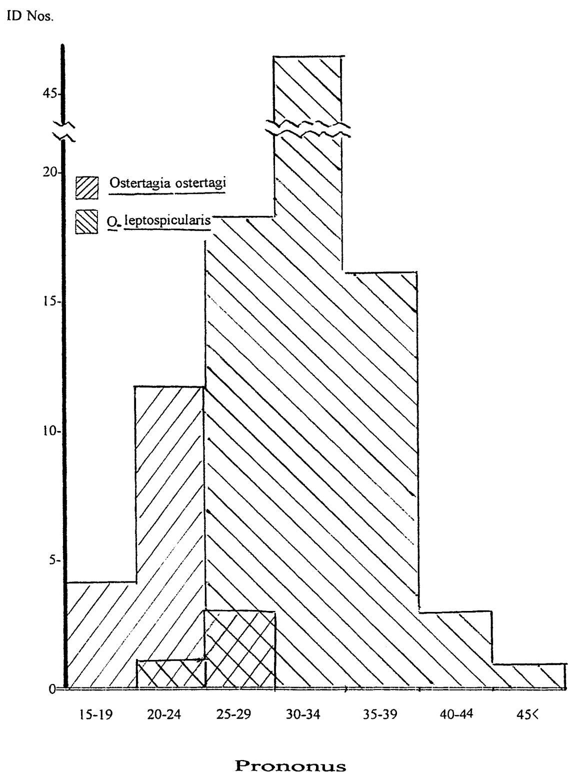

Fig. 15 Graph showing distribution of proconus height in *Ostertagia ostertagia* and *O. leptospicularis* from Chinese Water Deer.

zoos.

(ii) *Spiculopteragia asymmetrica.*

The genus *Spiculopteragia* is more prevalent in wild red, fallow and sika deer although little is known of its life-cycle and pathogenecity [25]. *S. asymmetrica* has been recovered from farmed red deer in East Anglia and may cause a Type II ostertagiosis [3].

The present study revealed that same six species of nematode were recovered from the abomasa of Chinese Water Deer that were reported by Ohira et al. [27] also working with Chinese Water Deer at WWAP. It is likely that *O. leptospicularis* and its minor morphotype, *S. kolchida*, were derived from other ruminabnts grazing at Whipsnade, rather than China, since these two parasites are confined to the western Palearctic and occur in cervids and bovids [14]. Whilst *O. ostertagi* was identified from animals in the present study, the "minor morphotype" of this parasite, *Skrjabinagia (Ostertagia) lyrata* [14, 18] was not. This may be because *O. ostertagi* normally occurs at low levels, in both numerical and prevalence terms in wild bovid and cervid hosts [14] or that Chinese Water Deer are not the parasite's primary host. Other species obtained from the Chinese Water Deer in this study on, *Camelostrongylus mentulatus* and *Trichostrongylus axei*, have been associated with a wide range of not only wild but also domestic camelids and/or bovids [29, 30, 32]. Since both these nematodes were recorded in an earlier at WWAP by Ohira et al. [27], both appear to be well-established in the Chinese Water Deer. Although *Spiculopteragia asymmetrica* was recovered by both Ohira et al. [27] and the present author, it was not possible to determine whether this parasite was a primary parasite of Chinese Water Deer or merely a secondary (or accidental) parasite of this host species. This parasite is regarded as a primary parasite of Palearctic cervids, such as *Cervus elaphus, C. dama, C. nippon* and *Capreolus capreolus* by Skrjabin et al. [30] and Hoberg et al. [14].

In the present study, *Ostertagia leptospicularis* and *Skrjabinagia kolchida* were not only the most numerous but also the most prevalent nematode species recovered from Chinese Water Deer. This is unlike the situation in domestic cattle (*Bos taurus*) where *S. kolchida* tends to be much less common. For example, Mulrooney et al. [24] found *S. kolchida* in only 18% of Oregon calves and that the mean burden was two worms. In contrast, the mean burden and prevalence of *O. leptospicularis* were 88 and 73%, respectively. *S. kolchida* has therefore tended to be regarded as the minor "minor morphotype" of *Ostertagia leptospicularis* [14, 18]. It is remarkable that the prevalence of *S. kolchida* is relatively high in Chinese Water Deer in the present study; indeed, one animal harbouring *S. kolchida* had no *O. leptospicularis* (deer ID No. 12) (Table 2). The prevalence of *O. ostertagi* in this study (67%) was higher than the 14% reported by Ohira et al. [27] thouygh less than the prevalence in domestic cattle (100%) [24]. The number and prevalence of other parasite species suggests that they are minor nematode species of the abomasum in Chinese Water Deer.

Previous work reported by Mulrooney et al. [24] indicated that the mean prevalence of two polymorphic species pairs was 96.1% *O. leptospicularis*: 3.9% *S. kolchida* and 99.7% *O. ostertagi* : 0.3%. However, in this study, the corresponding prevalence figures were more evenly balance at 64.6% *O. leptospicularis*: 35.4% *S. kolchida*. Clearly, the not only the intensity but also the prevalence of *S. kolchida* in Chinese Water Deer at WWAP is remarkable high. The factors responsible for this phenomenon require further investigation.

In the present study, approximately 17.9% of male *Ostertagia* spp. nematodes observed were identified as *O. ostertagi* according to standard identification keys [30, 34] (Fig. 9). According to these keys, the two *Ostertagia* species recorded in the present study may be differentiated by examination of the proconus which is well-developed (with an acute-angled bursa; Fig. 2) in the case of *O. leptospicularis* and less well developed (with a rounded bursa; Fig. 5) in the case of *O. ostertagi*. However, in this study, bursal shape and proconal size of the ostertagid nematodes appeared to be somewhat variable. For example, some *O. leptospicularis* had a slightly smaller proconus contained within a well-developed bursa (Fig. 3) while others had a well-developed proconus within a slightly rounded bursa (Fig. 4). Conversely, some individual *O. ostertagi* had a reasonably well-developed proconus (Fig. 6) in a more acute-angled bursa (Fig. 7). Therefore, a number of morphological measurements were made including the height of the proconus and the length of the bursa, the body, spicule and oesophagus for both *Ostertagia* spp. and *Skrjabinagia kolchida*, the minor morphotype of *O. leptospicularis* [18, 19], in order to evaluate the relative value of each as identification criteria.

Proconus. According to the 95% CI, this study confirmed that the height of the proconus of *O. leptospicularis* was larger than that of *O. ostertagi*, although there was some overlap in the range of values measured for the two worm species (Table 4, Figs. 10 and 15). This morphological feature may therefore be used as an aid to the identification of *O. leptospicularis* and *O. ostertagi*.

Bursa. Bursal length was found to be fairly constant amongst the *Ostertagia* spp., though that of *Skrjabinagia kolchida* was slightly larger (Tab. 4, Fig. 12) because there was a gap in the 95% CI between *Ostertagia* spp. and *S. kolchida*. Therefore, bursal size is of no value as an identification criterion for distinguishing between the *Ostertagia* species and *Skrjabinagia kolchida*.

Sjoberg's organ. *Skrjabinagia kolchida* was easily differentiated from the *Ostertagia* spp. by the presence of Sjoberg's organ (Figs. 1 and 8) and the absence of a well-developed proconus. Furthermore, it was evident that there was remarkable variation in the height of the genital cone and Sjoberg's organ within this species because the SDs were 11.64μm in the genital cone and 26.90μm in Sjoberg's organ, respectively (Tab. 4).

Other measurements. Conversely, there were no obvious differences in the length of the body, oesophagus or spicules between the two *Ostertagia* spp. becaues there were overlap between the 95% CI in these measurements between both species (Tab. 4, Figs. 11 and 13). However, oesophageal and bursal length of *S. kolchida* were significantly longer than the corresponding values for *O. leptospicularis* and *O. ostertagi*.

Conclusions

Whilst the six nematode species reported from the abomasum of Chinese Water Deer were the same as those listed by Ohira et al. [27], this is the first report of the relative numbers of each of these species in this host. *Ostertagia leptospicularis* and *Skrjabinagia kolchida* were the dominant species in Chinese Water Deer both in numerical and prevalence terms. It was also remarkable that the prevalence and intensity of infection with *S. kolchida* was relatively high in Chinese Water Deer (64.6% *O. leptospicularis*: 35.4% *S. kolchida*) compared with the relative numbers that have been reported from domestic cattle. Precisely why there were such large numbers is not known. This study also confirmed that measurement of the proconus could be used as a valuable criterion in the identification of *O. leptospicularis* and *O. ostertagi*.

However, before using criterion, more investigation about the other host species and worms populations including experimental hybrids [34]. The values of the other measurements, eg., bursa, body, spicule and oesophagus between *Ostertagia* spp. are almost constant, but ones of *Skrjabinagia kolchida* were larger than ones of *Ostertagia* spp.. This phenomenon is interesting because *S. kolchida* is the minor morphotype of *O. leptospicularis*. It is remarkable that there is variation of the heights of genital cone and Sjoberg's organ in *S. kolchida*. Both phenomena should be reinvestigated with regard to several matters, eg., nematode growth, hybridizations, host species, environments and so on, in future.

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要　　旨

英国ウイップスネード野生動物公園内に生息するシカ科動物キバノロ(*Hydropotes inermis*)オステルタジア亜科雄線虫の形態学的検討

シカ科動物キバノロ(*Hydropotes inermis*)は中国・朝鮮半島の湿原地帯に自然分布するが，ロンドン動物学会のウイップスネード野生動物公園(ロンドン北方約 40 km のイングラント地方に所在)内にも半野生下の状態で多数生息する。本研究はこのキバノロの第 4 胃に寄生するオステルタジア亜科線虫(家畜で病原性の高い種を含む)について検討した。検討項目は全般的な線虫相，*Ostertagia leptospicularis* と *Skrjabinagia kolchida* の各宿主個体における出現数比率，交接嚢およびそのほかの雄生殖器(proconus など)の形態的多型を応用したオステルターグ胃虫 *Ostertagia ostertagi* と *O. leptospicularis* との簡便な鑑別法であった。今回の検討では 6 種(*Camelostrongylus mentulatus, Ostertagia leptospicularis, O. ostertagi, Skrjabinagia kolchida, Spiculopteragia asymmetrica, Trichostrongylus axei*)，計 701 個体の線虫が得られた。これらにうち *O. leptospicularis* と *S. kolchida* とが寄生率および寄生数とも他種を凌駕した。これらに次いで，オステルターグ胃虫が検出されたので，反芻類家畜などへの本線虫種の媒介という側面からキバノロは警戒すべきであることが指摘された。出現比 *O. leptospicularis*：*S. kolchida* は 64.6：35.4 で，ほかの反芻類における値に比して後種の出現比が高かった点も注目された。また，*Ostertagia* 属 2 種および *S. kolchida*(この種は *O. leptospicularis* の一 morphotype とされる)で交接嚢など雄生殖器に大きさや形態に若干の変異が認められた。そこで，今後の疫学調査において簡便な鑑別方法を確立する目的で形態情報の整理を行うため，proconus 高，交接嚢高，生殖円錐高，Sjoberg's organ 高，体長，交接刺長，食道長について測定した。これらのうち，proconus 高に関しては *O. leptospicularis* の方がオステルターグ胃虫のものより発達している傾向が認められたので，この性質は鑑別指標としての候補となりうると考えられた。*S. kolchida* の生殖円錐と Sjoberg's organ は個体間で変異が著しいことが明らかにされた。本論文は，2001 年 9 月，著者がロンドン大学 Royal Veterinary College とロンドン動物学会とが共同開講する専門職大学院 Master of Science in Wild Animal Health(野生動物医学)に在学中，学位認定のため Royal Veterinary College に提出された Project Report を一部改稿したものである。

Summary

This study was designed to add to our knowledge of ostertagiines of captive Chinese Water Deer (*Hydropotes inermis*) focussing in particular upon two species that exhibit polymorphism in domestic ruminants because there has not been record from the host species. The objectives of the present study were to: -identify the genera and species of abomasal nematodes recovered at post mortem from Chinese Water Deer at Whipsnade Wild Animal Park,; -compare the relative numbers of *Ostertagia leptospicularis* and *Skrjabinagia kolchida* in each host; and -evaluate the measurement of the proconus as an aid to the identification of *Ostertagia leptospicularis* from *O. ostertagi*. A total of 701 nematodes recovered were examined in this survey, and these belonged to six species, i.e. *Camelostrongylus mentulatus, Ostertagia leptospicularis, O. ostertagi, Skrjabinagia kolchida, Spiculopteragia asymmetrica* and *Trichostrongylus axei*. *O. leptospicularis* and *S. kolchida* were not only the most numerous but also the most prevalent. Behind this, there are intensity and prevalence of *O. ostertagi*. Ratio of *Ostertagia leptospicularis* : *Skrjabinagia kolchida* was more evenly balance at 64.6% *O. leptospicularis*: 35.4% *S. kolchida*. It is remarkable that the prevalence of *S. kolchida* is relatively high in Chinese Water Deer. The not only the intensity but also the prevalence of *S. kolchida* *i*n Chinese Water Deer at Whipsnade Wild Animal Park is remarkable high. The factors responsible for this phenomenon require further investigation. In this study, bursal shape and proconal size was somewhat variable. Hence, the measurements of height of proconus and bursa with body, spicule and oesophagus of

Ostertagia spp. including *S. kolchida*, which is considered as morphotype of *O. leptospicularis* were done. In general, height of proconus of *O. leptospicularis* was larger than one of *O. ostertagia*, but bursal height is almost constant in *Ostertagia* spp.. Probably, this characteristics will be applied for one of new criteria of the identification of these *Ostertagia* species. There is a remarkable variation of the heights of genital cone and Sjoberg's organ of *S. kolchida*. On the other hand, there is no evident differences in the values of the measurements of body, spicule and oesophagus between *Ostertagia* spp..

Appendix 1 Measurements of Ostertagia spp. obtained from individual Chinese Water Deer.

Nos.	Body	Spicule	Oesophagus	Proconus	Bursa	Worm ID	Host ID Nos.
1	550	36	66	ND	18	Oo	4
2	ND*	32	ND	23	ND	Oo	1
3	485	29	59	28	29	Ol	1
4	438	32	61	30	31	Ol	1
5	455	35	65	33	30	Ol	1
6	460	31	40	20	28	Oo	1
7	510	33	68	28	28	Ol	1
8	535	33	65	38	29	Ol	1
9	550	35	58	29	33	Ol	1
10	460	32	55	19	30	Oo	1
11	585	33	69	29	38	Ol	1
12	496	30	42	28	30	Ol	1
13	570	35	68	28	33	Ol	1
14	565	38	60	34	33	Ol	4
15	520	36	67	35	28	Ol	4
16	478	35	45	30	31	Ol	1
17	420	33	66	30	33	Ol	1
18	430	29	54	28	29	Ol	1
19	493	30	66	32	28.5	Ol	1
20	365	31	55	24	28	Oo	1
21	470	32	58	35	30	Ol	1
22	430	31	55	29	27	Ol	1
23	490	35	59	32	28	Ol	1
24	510	31	60	32	28	Ol	1
25	ND	32	ND	ND	ND	Osp	1
26	520	32	58	30	28	Ol	1
27	525	31	64	26	31	Oo	4
28	510	39	65	35	34	Ol	4
29	470	34	60	24	30	Oo	4
30	490	35	63	30	32	Ol	1
31	518	31	60	32	29	Ol	1
32	460	29	61	32	32	Ol	1
33	530	32	60	26	28	Ol	1
34	520	29	56	23	29	Oo	1
35	450	34	42	15	ND	Oo	1
36	430	33	55	35	25	Ol	1
37	430	33	50	28	28	Ol	1
38	480	33	65	30	31	Ol	1
39	490	29	60	27	23	Ol	1
40	240	30	55	35	26	Ol	1
41	310	32	53	30	27	Ol	1
42	555	35	55	35	33	Ol	1
43	245	33	55	35	25	Ol	1
44	490	33	65	40	32	Ol	1
45	500	30	65	21	29	Oo	1
46	500	33	65	25	31	Ol	1
47	510	30	57	31	29	Ol	1
48	485	31	60	26	29	Ol	1
49	505	31	61	30	34	Ol	1
50	265	31	60	30	26	Ol	1
50'	580	32	59	30	26	Ol	1
51	510	32	63	32	27	Ol	1
52	500	32	60	35	29	Ol	1
53	360	32	45	30	ND	Ol	1
54	460	29	59	31	25	Ol	1
55	450	29	50	27	29	Ol	1
56	460	29	58	28	ND	Ol	1
56'	350	29	50	24	24	Oo	1
57	545	33	63	30	34	Ol	3
58	530	32	61	30	26	Ol	3
59	460	45	65	20	35	Oo	3
60	560	38	69	40	31	Ol	3
61	490	36	70	30	38	Ol	3
62	460	37	63	30	35	Ol	3
63	555	38	70	35	38	Ol	3
64	550	35	67	30	35	Ol	3
65	560	36	70	30	30	Ol	3
66	490	34	61	32	32	Ol	2
67	490	33	78	30	33	Ol	2
68	450	37	65	35	33	Ol	2
69	520	38	65	20	35	Oo	2
69'	485	30	59	30	ND	Ol	2
70	555	37	68	30	29	Ol	5
71	525	32	64	30	33	Ol	5
72	530	36	64	22	31	Ol	5
73	300	33	55	35	ND	Ol	5
74	340	30	55	32	30	Ol	5
75	530	31	64	22	31	Oo	5
76	360	31	55	33	28	Ol	5
77	450	33	58	30	28	Ol	5
78	430	35	58	30	28	Ol	5
79	480	33	64	38	31	Ol	6

Appendix 1 (continued)

Nos.	Body	Spicule	Oesophagus	Proconus	Bursa	Worm ID	Host ID Nos.
80	ND	36	ND	30	ND	Ol	6
81	510	31	63	40	34	Ol	7
81'	385	35	53	25	ND	Oo	7
82	390	36	60	45	35	Ol	7
83	490	36	62	30	32	Ol	7
84	520	36	61	35	35	Ol	7
85	330	37	50	24	30	Oo	7
86	430	29	58	37	30	Ol	8
87	555	33	66	32	30	Ol	9
88	465	29	67	32	27	Ol	9
89	515	32	62	30	30	Ol	9
90	524	32	63	30	30	Ol	9
91	555	35	64	33	35	Ol	9
92	475	35	63	20	31	Oo	9
93	480	29	59	28	27	Ol	9
94	480	30	62	30	29	Ol	9
95	560	35	66	37	32	Ol	9
96	510	35	60	32	ND	Ol	9
97	538	31	66	25	29	Ol	9
98	520	31	66	15	ND	Oo	9
99	560	36	65	30	29	Ol	10
100	490	30	62	25	25	Oo	11
101	500	33	61	29	32	Ol	11

* : If it was impossible to measure the structure, the item was recorded as ND -Not done.

Oo : *Ostertagia ostertagi*

Ol : *O. leptospicularis*

Osp: *O.* sp.

Appendix 2 Measurements of *Skrjabinagia kolchida* obtained from individual Chinese Water Deer.

Nos.	Body	Spicule	Oesophagus	(Sjoberg's organ)	Genial cone	Bursa	Host ID Nos.
102	505	30	55	(60)	ND*	27	1
103	465	35	68	(85)	ND	36	1
104	465	29	60	(46)	ND	28	1
105	540	32	73	(50)	14	34	1
106	480	28	56	(30)	13	30	1
107	365	30.5	55	(50)	ND	ND	1
108	520	34	68	(64)	ND	32	1
109	570	39	68	(80)	ND	38	1
110	440	34	60	(53)	ND	35	1
111	500	34	60	(70)	ND	ND	1
112	539	29	66	(52)	ND	34	1
113	520	35	64	(73)	15	38	1
114	300	31	50	(55)	15	32	1
115	430	29	40	(40)	15	32	1
116	360	37	60	(61)	15	35	1
117	350	34	60	(68)	ND	35	1
118	536	32	69	(75)	15	38	1
119	600	45	70	(130)	55	46	4
120	500	29	63	(44)	15	32	1
121	470	29	60	(40)	ND	29	1
122	500	31	ND	(60)	25	29	1
123	510	28	62	(43)	12	33	1
124	580	35	69	(82)	ND	40	1
125	360	33	65	(55)	ND	31	1
126	330	37	55	(56)	ND	37	1
127	585	30	57	(56)	ND	30	1
128	490	30	62	(61)	ND	34	1
129	530	29	57	(52)	ND	ND	1
130	590	42	72	(95)	15	44	3
131	605	43	69	(100)	ND	45	3
132	600	40	70	(95)	ND	ND	3
133	580	35	65	(80)	13	39	2
134	545	34	68	(85)	14	40	2
135	580	37	70	(85)	13	47	2
136	575	32	67	(55)	ND	31	5
137	550	35	64	(65)	15	39	5
138	545	36	63	(85)	ND	35	6
139	580	38	62	(90)	31	44	6
140	615	39	65	(110)	50	33	7
141	500	36	ND	(75)	15	37	9
142	560	34	66	(65)	15	37	9
143	626	35	67	(80)	35	41	9
144	520	30	56	(45)	10	33	9
145	ND	32	ND	(70)	ND	ND	9
146	535	32	62	(70)	ND	ND	9
147	500	35	62	(65)	15	39	10
148	440	34	58	(50)	ND	33	10
149	485	40	63	(90)	18	50	12

*: If it was impossible to measure the structure, the item was recorded as ND.